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SERUM ANTIBODIES TO HUMAN HEAT SHOCK PROTEIN 70 IN PATIENTS WITH CORONARY ATHEROSCLEROSIS

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Abstract: Previous studies suggest that antibodies to human heat shock protein 70 have a higher frequency in patients with risk factors for coronary atherosclerosis. We examined whether circulating anti-human heat shock protein 70 antibodies are associated with coronary artery disease. In a case control study preoperative blood samples for immunoblot analyses from 117 patients with severe coronary artery disease and 50 patients without coronary atherosclerosis were tested. Serum heat shock protein 70 antibodies were detectable in 8 patients undergoing bypass operations and one patient with aortic valve replacement. No association between anti-heat shock protein 70 IgG seropositivity and the prevalence of coronary artery disease was found ($p = 0.28$). These data provide evidence that anti human heat shock protein 70 IgG antibodies are not associated with coronary atherosclerosis.

Key words: heat shock protein 70, antibody, coronary atherosclerosis, cardiac surgery

1. Introduction

Autoantibodies against certain stress or heat shock proteins (HSP) may play a role in the pathogenesis of atherosclerosis.¹ Heat shock proteins are components of a physiologic stress response and Hsp70 is a highly conserved immunogenic molecule.² Many reports indicate that antibodies against HSPs are present in human serum.^{3,4,5}

In patients with risk factors for coronary atherosclerosis a higher frequency of antibodies against human HSP70 are documented. There was a statistical association of antibodies against Hsp70 with hypertension⁶ and hsp72 antibodies could be detected more frequently in smokers.⁷ Additionally HSP 70 antibodies were more often present among Type 1 diabetes subjects.^{8,9}

Therefore we tested the hypothesis if patients with severe coronary atherosclerosis undergoing CABG surgery, have a higher frequency of anti human HSP70 IgG antibodies.

2. Material and methods

2.1 Patients

The present study was reviewed and approved by the ethical committee of the University of Ulm. Analyses of antibody levels against human HSP70 were performed in serum from 167 subjects. Serum was obtained the day of admission from all patients undergoing elective cardiac surgery. The characteristics are given in Table 1.

2.2 Immunoblot Analysis

2.2.1 Serum Preparation

Circulating antibodies were determined in serum samples obtained from all study subjects after centrifugation at 4000 rpm for 4 min in a Labofuge GL (Heraeus Sepatech, Biofuge primo R) and stored at 4 °C.

Table 1.

Characteristics of the study subjects. ACB indicates aorto-coronary bypass; MVR mitral valve replacement/repair; AVR aortic valve replacement/repair; m male; f female; y years; m male; prot. protein; conc. concentration; mg milligram; ml millilitre;

Group	total n	ACB n	AVR/ MVR n
Patients	167	117	50
Age [y]	59.0 ± 18.4	65.7 ± 8.6	70.4 ± 9.1
sex [m/f]	119/48	93/24	26/24
prot. conc. [mg/ml]	31.3	32.6	30.1
anti-hHSP70	9	8	1

2.2.2 Membrane Preparation

A Hybond-PVDF membrane (Amersham Buchler Ltd) was preincubated in 100% methanol for 10 seconds, rinsed in aqua bidest for 5 minutes and blocked for 10 minutes in blocking buffer (70% aqua bidest, 20% methanol pure, 10% tris glycerine).

2.2.3 Protein Preparation

Protein was purchased by Stressgen. After different preparation steps protein was transferred to the membrane. The blot was blocked 1 hour in 5% nonfat milk solution (20 mmol/L Tris-HCl, 137 mmol/L NaCl, and 5% nonfat milk powder, pH 7.45), incubated with the 1:10 diluted (TTBS) patient serum for 30 minutes and washed six times for five minutes in 10 ml TTBS solution. Immunodetection of the serum antibodies was carried out with a 1:10 000 diluted peroxidase conjugated anti human IgG secondary antibody (NA 933 Amersham Ltd) for 30 minutes and washed again six times for five minutes in TTBS. After incubation with 0.1 ml/cm² ECL-plus detection reagent (Amersham buchler Ltd) for 5 minutes, blots were exposed to Hyperfilm ECL (Amersham Ltd) for 5 and 15 minutes. Protein concentration was determined according to Bradford.¹¹

2.2.4 Quantification of Immunoreactive Bands

After development the blots were scanned with a densitometer (Umax, Astra 6450, Fremont, CA; Canon, Cano-Scan1220 U), and the bands were analysed with a software program (mars 98, version 1.0.1). To determine the amount of antibody binding, a standard dilution with known antibody concentrations was prepared. A linear relationship was found between the known antibody concentrations and the densitometric integral ($r = 0.95$). On the basis of this linear relationship, anti HSP70 of each serum sample was calculated.

2.2.5 Statistical analysis

To detect differences between groups, the Fischer Exact test was performed. The $P < 0,05$ value was considered statistically significant. The correlation between standard HSP and the densitometric integral was examined by linear regression analysis.

3. Results

3.1 Antibody frequency in the sera of the subjects

In order to assess whether there were any changes in antibody status, immunoblot analysis was carried out from 117 patients with angiographic evidence for severe coronary atherosclerosis and 50 patients without coronary atherosclerosis.

A total of 167 subjects were studied. Men constituted 66.8% and whites 100% of the cohort. Their ages ranged from 38-89 years. To demonstrate antibody reaction, repre-

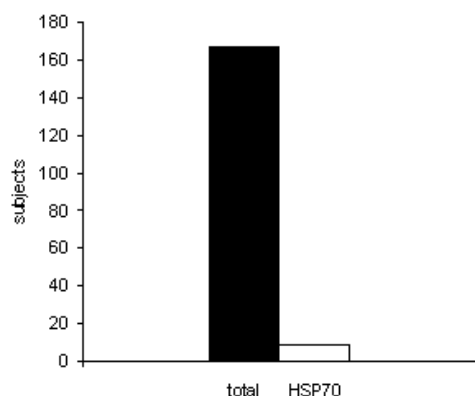


Fig. 2.
Number of HSP70 autoantibody positive patients. 9 positive samples from 167 sera tested.



Fig. 1.
Representative immunoblots from 4 different patient sera with membrane bound human HSP70 detected with horseradish peroxidase secondary AB, ECL-plus technic, exposed to hyperfilm.

sentative immunoblots are shown in figure 1. Visual inspection of the antibody bands shows different band densities. Anti human HSP70 antibodies were found in 9 sera. 8 of them had coronary atherosclerosis and one patient underwent aortic valve replacement. Graphic illustration according figure 2, shows the HSP70 antibody positive sera from all patients.

If analysed according the underlying pathogenesis graphic representation shows that the majority of patients positive for human HSP70 derive from the coronary atherosclerosis group (Fig. 3). But statistical analysis demonstrate an insignificant distribution according disease groups. ($p = 0,28$).

4. Discussion

Heat shock or stress proteins are typically regarded as being intracellular proteins, which have a range of function including the maintenance of cellular integrity. Members of the Hsp70 family of molecules have been implicated in the processing and presentation of antigen, and the cross reactivity of lymphocytes specific for pathogen-derived heat shock proteins with self Hsp70 has been suggested to be an underlying cause of certain autoimmune diseases.

This study reports the presence of soluble anti-human Hsp70 IgG antibodies in the peripheral circulation of patients with and without coronary atherosclerosis. Circulating anti-human Hsp70 antibodies were detected in 5.4 % of all individuals examined.

Our findings support previous observations showing that there was no statistical significant difference in the level of anti-hsp70 antibodies between patients with severe CAD and controls.¹¹ Furthermore no association between anti-HSP70 IgG seropositivity and the prevalence of CAD was found.¹²

The physiological role for circulating anti-human HSP70 is currently unknown, but we suggest that humoral immunity against human HSP70 does not contribute to coronary atherosclerosis.

These findings support the view that atherosclerosis might not be a consequence of an anti-human HSP70 antibody induced development of atherosclerosis. Whether these

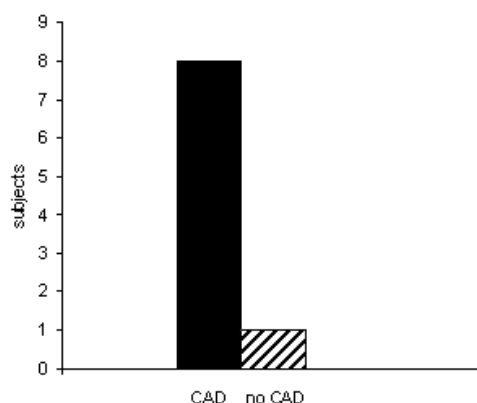


Fig. 3.
HSP70 autoantibody status in patients with and without CAD. 8 patients from the CAD group and one patient from the control group were positive for HSP70 auto antibodies. ($p=0.28$)

antibodies are of pathogenetic significance or may be used as a disease marker will have to be elucidated in future experiments.

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ANTIBODIES TO HUMAN HEAT SHOCK PROTEIN 60 AND MYCOBACTERIAL HEAT SHOCK PROTEIN 65 IN PATIENTS WITH CORONARY ATHEROSCLEROSIS

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Objective: In patients with coronary atherosclerosis, conflicting results related to the influence of heat shock protein antibodies exist. Our aim was to determine antibodies against human HSP60 and mycobacterial HSP65 in sera of patients with coronary atherosclerosis.

Methods: Immunoblot analyses of antibodies against human HSP60 and mycobacterial HSP65 were performed in serum from 117 patients with coronary atherosclerosis and 50 patients without angiographic evidence of coronary atherosclerosis.

Results: Anti human HSP60 autoantibodies were found in 8 patients undergoing CABG ($p=0,11$). Anti mycobacterial HSP65 antibodies were found in six CABG patients and one patient without coronary atherosclerosis ($p=0,68$).

Conclusions: This result is consistent with a previous report, suggesting that antibodies against human HSP60 or mycobacterial HSP65 may not be involved in coronary atherosclerosis.

Key Words: Atherosclerosis, heat shock protein 60/65, immunoblot

1. Introduction:

In the multifactorial pathogenesis of atherosclerosis, antibodies to the 60 kD heat shock protein (HSP) class have been demonstrated. Elevated levels of mycobacterial HSP65 (mHSP65) antibodies were reported in patients with coronary atherosclerosis [1,2]. Also a strong association has been found between high anti mHSP65 levels and restenosis after PTCA [3]. Moreover mHSP65 antibody titers were higher in patients with future cardiovascular events [4].

Additionally, in patients with coronary atherosclerosis high levels of auto antibodies against human HSP60

(hHSP60) were found [5]. They are considered an independent, novel family risk factor for severe coronary atherosclerosis [6]. Furthermore levels of complement activating anti-hHSP60 antibodies are elevated in atherosclerosis related disease [7].

In contrast to these results, a previous investigation showed that serum IgG antibodies to human HSP60 are not associated with CAD [8]. The aim of the present study was therefore to determine antibodies against mycobacterial HSP65 and human HSP60 in sera of patients with and without angiographic evidence of coronary atherosclerosis.